REMARKS

This is a response to the Office action dated September 30, 2009. Claims 1-2, 4-13, and 15-27 are pending and at issue. Claim 14 is pending but remains withdrawn from consideration.

No new matter has been added.

Reconsideration of the present application is respectfully requested.

Claims 1, 2 and 4-27 are pending. Claims 1, 5, 7, 12-15, 17, 25-27 have been rejected as allegedly being anticipated by the prior art. Claims 1, 2 and 4-27 have been rejected as allegedly being obvious over the prior art.

Rejection Under 35 U.S.C. §102(b) (anticipation)

The Examiner asserts that claims 1, 5, 7, 12-15, 17 and 25-27 are anticipated by Chauhan et al., *Gene* 120:281-286 (1992) ("Chauhan"). The Examiner's position is that the vector pSSC-9 disclosed in Fig. 1 of Chauhan has the same arrangement of a gene, DNA sequence and promoter as recited in the claims, that Chauhan discloses a method of inserting the vector into a cell, and that "[h]omologous recombination would occur between the homologous DNA sequences." *See* Office Action at pages 2-3, bridging paragraph.

Respectfully, the Examiner's assertion that Chauhan anticipates the claims is not correct. Chauhan's vector pSSC-9 is comprised of a neomycin resistance (neo^r) gene flanked by thymidine kinase (tk) genes. Each of these genes is controlled by its own tk promoter. The neo^r gene is flanked by restriction sites useful for the cloning of additional sequences, i.e., sequences derived from a gene targeted for disruption by homologous recombination.

pSSC-9 was designed to select for gene disruptions due to homologous recombination at the sequences flanking the neo^r gene (by simultaneous positive selection for the neo^r gene present between the flanking sequences and negative selection for he tk genes that are on either side of the flanking sequences). *See* Abstract and page 283, column 1, lines 4-16. However, the sequences flanking the neo^r gene that undergo homologous recombination are not under the control of a promoter. Hence, the use of the pSSC-9 vector does not induce homologous recombination of a gene that is 3' to a promoter controlling transcription of the gene, as required by the claims. For this reason, Chauhan does not anticipate any of claims 1, 5, 7, 12-15, 17 and 25-27.

The Examiner, respectfully, is also incorrect to assert that Chauhan inherently discloses the claimed methods of enhancing homologous recombination. Chauhan, in fact, fails to disclose any homologous recombination event. As set out above, pSSC-9 is designed to allow and select for homologous recombination events that occur at additional sequences cloned into the sites flanking neo^r. Chauhan, however, reports on experiments designed to test the expression of the neo^r and tk genes after the parent vector pSSC-9, i.e., vector without any additional sequences flanking the neo^r gene, is transfected into cells. Following transfection of the parent vector, neither of the tk genes nor the neo^r gene undergoes a homologous recombination.

Chauhan thus reports that although transfection of either ES or Y1 cells with pSSC-9 yielded colonies that were resistant to gentamycin, "selection of either cell type in the presence of Gt [gentamycin] and 10 μm Gc resulted in no cell survival (Fig. 2)." *See* Chauhan at page 283, Experimental and Discussion, paragraph (b) Activity of selectable markers (*tk* and *neo*^{R}) (emphasis added). Thus, as expected, Chauhan obtained zero homologous recombinants using the parent vector pSSC-9. Chauhan thus does not disclose the claimed method of inducing homologous recombination (because no homologous recombination has taken place). For this additional reason, Chauhan does not anticipate the claimed methods.

Rejections Under 35 U.S.C. §103(a) (obviousness)

Claims 1, 5, 7, 12-15, 17 and 25-27 are rejected as allegedly obvious over Nickoloff, *Mol. Cell. Biol.* 12:5311-5318 (1992) ("Nickoloff"). Applicants note that Nickoloff was cited by the Examiner in a previous Office Action as an anticipating reference. The anticipation rejections have been overcome. The claims recite that homologous recombination is induced by controlling transcription of a gene using a transcription promoter that is located 3' to the DNA sequence and that the gene is located 3' to the transcription promoter. Nickoloff's vector the MMTV promoter is placed at the 5' end of and regulates transcription of the first of the two neo genes (the "upstream" neo gene). Thus, Nickoloff does not anticipate because the MMTV promoter is not placed between the two neo genes and does not regulate transcription of the second (downstream) neo gene.

The Examiner now takes the position that it would have been obvious to enhance homologous recombination by modifying Nickoloff such that a promoter is used to regulate transcription of the downstream gene. According to the Examiner, Nickoloff discloses that transcription enhances recombination between direct and inverted repeats and requires transcriptional activity in only one repeat, and that recombination is enhanced when both repeats are transcriptionally active. The Examiner asserts it therefore would have been obvious to one of ordinary skill in the art that transcription of either neo gene would be encompassed and that due to the high level of skill in the art and absent evidence to the contrary, there would have been a reasonable expectation of success to obtain the claimed invention.

Respectfully, the Examiners conclusion is not well-taken. It would not be obvious to modify Nickoloff by moving the MMTV promoter to regulate the downstream neo gene. Obviousness requires a reasonable expectation that the prior art could be successfully modified to arrive at the claimed invention. Here, the Examiner bases a reasonable expectation of success

on the assertion that Nickoloff "discloses that transcription enhances recombination between direct and inverted repeats and requires transcriptional activity in only one repeat, and when both repeats are transcriptionally active." Applicants submit, however, that the Examiner misinterprets the meaning of this passage.

Within the context of Nickoloff, one of ordinary skill in the art would recognize the statement that enhanced recombination "requires transcriptional activity in only one repeat" means only that transcription of the upstream neo gene is itself sufficient to enhance recombination. Moreover, each of the constructs disclosed in Nickoloff includes a transcriptionally active upstream neo gene. Nickoloff thus fails to include any indication that transcription of the downstream neo repeat would have the effect of enhancing homologous recombination. In the absence of such evidence, applicants submit that there is no expectation that homologous recombination would be enhanced by transcription of the downstream neo gene.

Nor does the simple, unsupported statement that "[p]reliminary data indicate that similar effects are found when both repeats are transcriptionally active" change this analysis, because this arrangement would still include transcription of the upstream neo gene. Consequently, the claims are thus not obvious over Nickoloff because there is no reasonable expectation that Nickoloff could be successfully modified to arrive at the claimed invention.

As can be also understood from the experimental data in the specification of the present invention, the recombination frequency was ultimately increased up to approximately 0.3% by using the present invention, (equivalent to an increase in frequency that is 1000-fold the frequency of a spontaneous recombination) whereas the recombination frequency of Nickoloff et al. is two orders of magnitude lower at approximately 10-5, and the recombinant activity

associated with the Nickoloff transcriptional induction is only a few times greater (low S/N ratio, see TABLE 2 of Nickoloff et al.). This is certainly indicative of a difference in kind.

Moreover, while the present invention allows for the results of an assay of homologous recombinant efficiency to be obtained within an optimum of 4 days from the start of transcriptional induction, Nickoloff et al. requires approximately 11 days from the start thereof, which is three times as long (please refer to the page on "Recombination assay" in the "MATERIALS AND METHODS" of Nickoloff et al.). Thus, the method(s) described in Nickoloff et al. would induce recombination at an extremely low frequency when compared to the method(s) of the present invention.

Considering the differences in the abovementioned results, the present inventors believe that since the transcription promoter is positioned as the most upstream element out of all the elements in Nickoloff et al., it has the effect of loosening the overall chromatin structure of the downstream region. The present inventors believe further that the low level of DNA homologous recombination (TABLE 2) is a result of this loosening. In addition, by employing such a configuration, the method of Nickoloff et al. causes other problems, even with regard to the direction of the recombinant DNA. Specifically, the "inverted-repeat" type in Nickoloff et al., which has an orientation that is similar to that of the present invention with respect to target gene-template gene (Fig. 6), results in various types of recombinants such as a chiasma or unequal chiasma that do not have a shape that would be induced by unidirectional genetic conversion alone. On the other hand, in so far as the recombinant obtained in the present invention has been studied, even without the presence of a particular selective force, since there are no adverse effects caused by the transcription, the overall genetic conversion is unidirectional, and thus the present method has practical applicability.

As explained above, applicants submit that those skilled in the art could not have conceived of the constitution of the present invention, which promotes recombination at a frequency so unpredictably high that it reaches a practical level, based on the structure and effects shown by Nickoloff et al.

The Examiner has made additional rejections of claims 2, 8, 18 and 23 as obvious over Nickoloff in view of Lahti, *Methods* 17:305-312 (1999) ("Lahti"), claims 9, 10, 19 and 20 as obvious over Nickoloff and Slebos et al., *Biochem. Biophys. Res. Comm.* 281:212-219 (2001) ("Slebos"), claims 4, 6, 11, 16, 21 an 24 as obvious over Nickoloff in view of Phi-van et al., *Biochem.* 35:10735-10742 ("Phi-van") or Israel et al., *Nucl. Acids Res.* 17:4589-4604 ("Israel") and claim 22 as obvious over Nickoloff in view of Phi-van or Israel and Lahti.

Each of the additional rejections relies on Nickoloff as the primary reference and requires that Nickoloff be modified to rearrange placement of a promoter to arrive at the rejected claims. For the reasons discussed above, it is our opinion that, contrary to the Examiner's position, Nickoloff fails to provide either a motivation or reasonable expectation of success to arrive at the instant claims. (Nor does Nickoloff obtain the resulta of the present invention.) The Examiner cites the secondary references for the proposition that they recite or suggest the limitations of DT40 cells (Lahti), easily assayable genes such as ECGP or EGFP (Slebos) and MAR or enhancer elements (Phi-van and Israel). The secondary references thus fail to provide the motivation or expectation of success that is lacking in Nickoloff. Thus, the additional rejections, in our opinion, should also be withdrawn because the various combinations of reference cited by the Examiner still fail to suggest modifying the prior art (i.e., Nickoloff) to place a promoter element such that it drives expression of a downstream element in a homologous recombination event.

If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Respectfully submitted,

MERCHANT & GOULD P.C.

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By _____
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